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Isolation of *Schizosaccharomyces pombe* Isopentenyl Diphosphate Isomerase cDNA Clones by Complementation and Synthesis of the Enzyme in *Escherichia coli**

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Isopentenyl diphosphate (IPP) isomerase catalyzes an essential activation step in the isoprene biosynthetic pathway. The *Saccharomyces cerevisiae* gene for IPP isomerase, *IDI*1, was recently isolated and characterized (Anderson, M. S., Muehlbacher, M., Street, I. P., Proffitt, J., and Poulter, C. D. (1989) *J. Biol. Chem.* 264, 19169–19175), and the wild-type gene, *IDI*1, was disrupted with a *LEU*2 marker to create a diploid yeast strain heterozygous for the *idi*1::*leu*2 disruption, which revealed that *IDI*1 was an essential single-copy gene (Mayer, M. P., Hahn, F. M., Stillman, D. J., and Poulter, C. D. (1992) *Yeast* 8, 743–748). We now report the isolation of a cDNA clone from *Schizosaccharomyces pombe* by a plasmid shuffle-mediated complementation of the *LEU*2 disrupted yeast gene. The *S. pombe* clone encoded a 26,864-dalton polypeptide of 227 amino acids with a high degree of similarity to the *S. cerevisiae IDI*1 enzyme. *S. pombe* IPP isomerase contained the essential Cys and Glu catalytic residues identified in yeast isomerase (Street, I. P., Coffman, H. R., Baker, J., and Poulter, C. (1994) *Biochemistry* 33, 4212–4217) but was significantly smaller than the *S. cerevisiae* enzyme. The plasmid shuffle technique is an excellent procedure for screening expression libraries for IPP isomerase activity by complementation of the *idi*1 mutation.

The isoprenoid biosynthetic pathway, with more than 23,000 known metabolites, is the most diverse found in nature. The pathway is found in all organisms (1) and produces several important classes of compounds, including sterols (2), carotenoids (3), dolichols (4), ubiquinones (5), and prenylated proteins (6). All of these compounds are derived from linear isoprenoid diphosphates synthesized from the isomeric five-carbon intermediates isopentenyl diphosphate (IPP)[1] and dimethylallyl diphosphate (DMAPP) (7). In eukarya and archae where the pathway is well established, IPP is synthesized from three molecules of acetyl-CoA by the mevalonate pathway (8). However, in some bacteria IPP appears to be synthesized from an as yet unidentified three-carbon precursor by a new non-mevalonate route (9). Beyond IPP, the pathway for constructing polyisoprenoid chains is similar for organisms in all three major kingdoms.

Isopentenyl diphosphate:dimethylallyl diphosphate isomerase (IPP isomerase; EC 5.3.3.2) catalyzes a crucial activation step in the isoprenoid pathway by converting IPP to its highly electrophilic isomer DMAPP. These two intermediates are the initial substrates for prenyltransferases that synthesize polyisoprenoid chains. The antarafacial isomerization of IPP to DMAPP illustrated in Scheme 1 (7) occurs by a protonation-deprotonation mechanism (10–13).

*IDI*1, the gene for IPP isomerase in *Saccharomyces cerevisiae*, was recently isolated (14) and characterized as a single-copy gene on chromosome XVI (15). Recombinant yeast enzyme was purified to homogeneity, and two essential catalytic site residues, Cys^{139} and Glu^{207}, were identified by a combination of affinity labeling and site-directed mutagenesis experiments (16–18). Recently, Xuan *et al.* (19) reported a human cDNA clone with an open reading frame (orf) that encoded a protein with a high degree of similarity to yeast IPP isomerase, including the essential Cys and Glu residues. However, the putative human enzyme had a 52-amino acid deletion at its amino terminus, and catalytic activity was not established. We were interested in developing a procedure that would allow us to screen heterologous libraries for genes encoding IPP isomerase by complementation in order to obtain sequence information and protein for comparative studies. We previously described construction of *S. cerevisiae* strain FH2-5b (15) and now report its use to isolate a cDNA clone for IPP isomerase from *Schizosaccharomyces pombe* by a plasmid shuffle technique and synthesis of the *S. pombe* enzyme in *Escherichia coli*.

EXPERIMENTAL PROCEDURES

Materials—[1-^{14}C]IPP was purchased from Amersham Corp. DE52 ion exchange resin was from Whatman, and phenyl-Sepharose was purchased from Pharmacia Biotech Inc. DL-α-Aminoadipic acid (α-AA), adenine, uracil, L-leucine, L-lysine, L-tryptophan, and L-histidine were from Sigma. SeaKem ME agarose was from FMC BioProducts. All restriction endonucleases were purchased from New England Biolabs, and T4 DNA ligase was from Boehringer Mannheim. pBluescript II SK(+) was from Stratagene. pARC306N was provided by M. Bittner (Biotechnology Division, Amoco Research, Naperville, IL). pIPS139 was available from a previous study (18). pYES2.0 was from Invitrogen. Oligonucleotide primers and linkers were synthesized by Bob Schackmann, Utah Regional Cancer Center, Protein/DNA Core Facility.

Strains, Media, and Growth Conditions—The haploid *S. cerevisiae* strain FH2-5b (*MAT*α, *ade*2-101, *his*3-Δ200, *leu*2-Δ1, *lys*2-801, *trp*1-Δ63, *ura*3-52, *idi*1::*LEU*2, pRS317:*IDI*1) was described by Mayer *et al.* (15). A *S. pombe* cDNA expression library was obtained from L. Guarente, Harvard University (20). *E. coli* strains DH5α (F$^-$, φ80d*lac*ZΔM15, *end*A1, *rec*A1, *hsd*R17 (r^-_k, m^-_k), *sup*E44, *thi-1*, λ$^-$, *gyr*A96, *rel*A1; Life Technologies, Inc.) and JM101 (Δ(*lac-pro*AB), *sup*E, *thi*/F′, *tra*D36. *pro*AB, *lac*I^{q}ZΔM15 (21) were used for cloning procedures. *S. cerevisiae* strains were grown at 30 °C in YEPD medium (22) or synthetic minimal medium (23, 35) plus one or more of the following

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U21154.

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[1] The abbreviations used are: IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; orf, open reading frame; α-AA, DL-α-aminoadipic acid; kb, kilobase(s); BME, β-mercaptoethanol.

supplements as required by auxotrophies: adenine (0.06 g/liter), uracil (0.06 g/liter), leucine (0.1 g/liter), lysine (0.08 g/liter), tryptophan (0.08 g/liter), and histidine (0.02 g/liter). Medium containing α-AA is described by Sikorski and Boeke (24). Transformed *E. coli* strains were grown at 37 °C in LB broth containing 5 g of Difco yeast extract, 10 g of Difco tryptone, 10 g of NaCl, and 75 mg of ampicillin in 1 liter of H_2O or M9/CAGM medium containing M9 salts (25), casamino acids (1% w/v), glucose (0.26% w/v), $MgSO_4$ (0.3 g/liter), $CaCl_2$ (0.004 g/liter), thiamine hydrochloride (0.025 g/liter), $FeCl_2$ (0.0054 g/liter), and ampicillin (75 mg/liter). Radioactivity was measured in Optifluor scintillation fluid (Packard Instrument Co.) using a Packard Tri-Carb model 4530 liquid scintillation spectrometer.

General Methods—Minipreparations of plasmid DNA for restriction analysis were obtained by the boiling method as described by Sambrook *et al.* (25). Large scale plasmid preparations (>100 μg) were performed using a purification kit from Qiagen. DNA fragments were purified using a GeneClean kit from Bio101. Restriction digests, ligations, and *E. coli* transformations were conducted as described by Sambrook *et al.* (25). Plasmids were isolated from yeast as described by Robzyk and Kassir (26). IPP isomerase was assayed by the acid lability procedure (27). Ion exchange and hydrophobic interaction chromatographies were conducted at 4 °C using a Pharmacia FPLC system. SDS-polyacrylamide gel electrophoresis was performed using the discontinuous buffer system of Laemmli (28), and the gels were stained with Coomassie Brilliant Blue R (Sigma). Protein concentrations were determined by the method of Bradford (29) using bovine serum albumin as standard.

Transformation of S. cerevisiae Strain FH2-5b—*S. cerevisiae* strain FH2-5b was grown in YEPD medium to $OD_{600} \sim 0.75$ and transformed with 10 μg of DNA from the *S. pombe* library by the modified lithium acetate procedure of Elbe (30). Transformants were plated on ura$^-$, leu$^-$, lys$^-$ media (synthetic minimal media plus adenine, histidine, and tryptophan) and selected for prototrophy on media lacking the supplement corresponding to the selectable marker on the plasmid used for the transformation.

α-AA Selection/Counterselection—Plates containing *S. cerevisiae* transformed with the *S. pombe* cDNA library were washed with 5 ml of medium containing α-AA (24). The colonies were removed from the surface by gentle swirling, transferred to sterile 50-ml centrifuge tubes, and spun for 10 min at 3,000 rpm. The supernatants were decanted, and the pellets were resuspended in 1 ml of α-AA medium. The entire suspension was transferred to α-AA plates (100 μl/plate) and incubated at 30 °C.

Plasmid Isolation from α-AA Selected Yeast Colonies—Cultures (3 ml) containing α-AA (2 mg/ml) were prepared by inoculation with individual *S. cerevisiae* colonies that survived α-AA selection and incubated at 30 °C until turbid. Plasmid DNA was isolated and used to transform *E. coli* DH5α. Single colonies resulting from the transformation reactions were used to inoculate 3 ml of LB/ampicillin, and the cultures were grown overnight at 37 °C.

Complementation of Disrupted IDI1—The heterozygous diploid yeast strain FH1 (DY833 *idi1::leu2*) was transformed, as described in Mayer *et al.* (15). Transformants were used to inoculate sporulation media (23, 35) and grown at 30 °C for 5 days. The asci were dissected (23, 35) on a YEPD plate. Following incubation at 30 °C, spores that were both uracil and leucine prototrophs were identified by replica plating. Mating type determinations (31) were used to identify haploid spores as follows. A master plate corresponding to the dissected asci was created by streaking separated spores on a YEPD plate. Each horizontal row of streaks corresponded to a dissected ascus. Following overnight incubation at 30 °C, the master plate was replica plated to synthetic minimal medium. Each streak on the synthetic minimal plate was cross-streaked with both mating type **a** and mating type α strains, and the plate was incubated overnight at 30 °C. A patch of growth at cross-streaked intersections indicated a positive mating reaction.

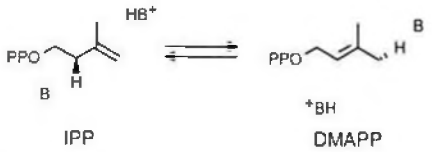
Cloning the S. pombe IPP Isomerase Gene—*Hin*dIII digests were performed on four plasmids identified as containing DNA able to complement disrupted *S. cerevisiae IDI1*. The 1-kb bands were excised from 0.8% agarose gels, purified by GeneClean, and ligated into the *Hin*dIII site of pBluescript II SK(+).

Sequencing Methods—DNA sequence analysis was performed by the dideoxy chain termination method of Sanger *et al.* (32) with a Sequenase 2.0 kit (U. S. Biochemical Corp.). Using primers corresponding to the T3 and T7 promoters of pBluescript, the four cDNA inserts were partially sequenced and determined to be identical. The insert in pBSF19 was sequenced in both directions. Computer-assisted analysis of sequence data was performed using MacGel 1.0, DNA Strider and Translate.

Expression and Purification of S. Pombe IPP Isomerase—Unique *Nde*I and *Sal*I restriction endonuclease sites were introduced into the *S. pombe* IPP isomerase gene in pBSF19 by Kunkel mutagenesis (33) using the Muta-Gene Phagemid *in vitro* Mutagenesis Kit version 2 (Bio-Rad) and primers 5′-CAGTGTGCTCTAC**ATATG**AGTTCCCAA-CAA-3′ and 5′-CGTTGC**TAA**AGTCGACAAATTTTACCAGAG-3′. Start and stop codons are in bold, and the new restriction sites are underlined. A *NdeI/SalI* double digest of the mutants gave a 0.7-kb DNA fragment containing *S. pombe IDI1*. The DNA was electrophoresed on a 0.8% agarose gel, excised, purified by GeneClean, and ligated into the *Nde*I and *Sal*I sites of the *E. coli* expression vector pARC306N.

The resulting plasmid, pFMH6, was used to transform *E. coli* JM101 competent cells. LB/ampicillin cultures (3 ml) were inoculated with single colonies and grown overnight at 37 °C. The 3-ml cultures were then used to inoculate 330 ml of M9/CAGM. The cultures were incubated at 37 °C until the OD_{600} was 0.6–0.9. Nalidixic acid (15 mg) was added, and incubation was continued for 3 h. Cells were harvested by centrifugation at 10,000 × *g* for 10 min and frozen at −80 °C in 1-g portions.

Two grams of cell paste were suspended in 10 ml of 10 mM potassium phosphate, 10 mM β-mercaptoethanol (BME), 1 mM phenylmethylsulfonyl fluoride, pH 7.0, and disrupted by sonication. The resulting homogenate was centrifuged at 4 °C to remove cellular debris. The supernatant was then loaded at 4 °C on a 1.5 × 30-cm DE52-cellulose column previously equilibrated with 10 mM potassium phosphate, 10 mM BME, pH 7.0 (buffer A). The column was eluted at 1.5 ml/min with a 150-ml linear gradient of buffer A to 100% buffer B (500 mM potassium phosphate, 10 mM BME, pH 7.0). Active fractions were collected and com-



SCHEME 1. **Interconversion of IPP and DMAPP.**

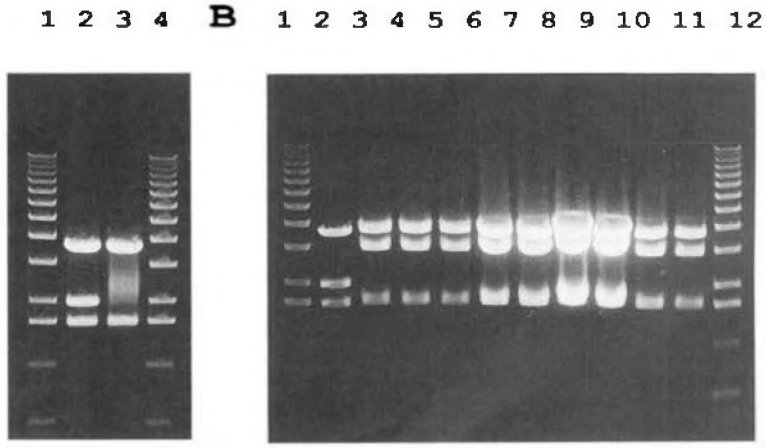


FIG. 1. **Restriction analysis of pDB20, *S. pombe* cDNA library and plasmids isolated from a single α-AA-selected yeast colony.** *Panel A*: *lanes 1* and *4*, DNA markers; *lane 2*, *Bgl*I digest of pDB20; *lane 3*, *Bgl*I digest of *S. pombe* cDNA library. *Panel B*: *lanes 1* and *12*, DNA markers; *lane 2*, *Bgl*I digest of pDB20; *lanes 3–11*, *Bgl*I digests of isolated plasmids from a single survivor of α-AA counterselection.

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GCGGCCGCCAGTGTGCTCTAAAGATGAGTTCCCAACAAGAGAAAAAGGATTATGATGAAG 60
                                M  S  S  Q  Q  E  K  K  D  Y  D  E  12

AACAATTAAGGTTGATGGAAGAAGTTTGTATCGTTGTAGATGAAAATGATGTCCCTTTAA 120
E  Q  L  R  L  M  E  E  V  C  I  V  V  D  E  N  D  V  P  L   32

GATATGGAACGAAAAAGGAGTGTCATTTGATGGAAAATATAAATAAAGGTCTTTTGCATA 180
R  Y  G  T  K  K  E  C  H  L  M  E  N  I  N  K  G  L  L  H   52

GAGCATTCTCTATGTTCATCTTTGATGAGCAAAATCGCCTTTTACTTCAGCAGCGTGCAG 240
R  A  F  S  M  F  I  F  D  E  Q  N  R  L  L  L  Q  Q  R  A   72

AAGAGAAAATTACATTTCCATCCTTATGGACGAATACATGTTGCTCCCACCCATTGGATG 300
E  E  K  I  T  F  P  S  L  W  T  N  T  C  C  S  H  P  L  D   92

TTGCTGGTGAACGTGGTAATACTTTACCTGAAGCTGTTGAAGGTGTTAAGAATGCAGCTC 360
V  A  G  E  R  G  N  T  L  P  E  A  V  E  G  V  K  N  A  A  112

AACGCAAGCTGTTCCATGAATTGGGTATTCAAGCCAAGTATATTCCCAAAGACAAATTTC 420
Q  R  K  L  F  H  E  L  G  I  Q  A  K  Y  I  P  K  D  K  F  132

AGTTTCTTACACGAATCCATTACCTTGCTCCTAGTACTGGTGCTTGGGGAGAGCATGAAA 480
Q  F  L  T  R  I  H  Y  L  A  P  S  T  G  A  W  G  E  H  E  152

TTGACTACATTCTTTTCTTCAAAGGTAAAGTTGAGCTGGATATCAATCCCAATGAAGTTC 540
I  D  Y  I  L  F  F  K  G  K  V  E  L  D  I  N  P  N  E  V  172

AAGCCTATAAGTATGTTACTATGGAAGAGTTAAAAGAGATGTTTTCCGATCCTCAATATG 600
Q  A  Y  K  Y  V  T  M  E  E  L  K  E  M  F  S  D  P  Q  Y  192

GATTCACACCATGGTTCAAACTTATTTGTGAGCATTTTATGTTTAAATGGTGGCAGGATG 660
G  F  T  P  W  F  K  L  I  C  E  H  F  M  F  K  W  W  Q  D  212

TAGATCATGCGTCAAAATTCCAAGATACCTTAATTCATCGTTGCTAAAGTCTTTAAATAT 720
V  D  H  A  S  K  F  Q  D  T  L  I  H  R  C  -               227

TACCAGAGATAAAGAAAAATAGAGCTATGAATTGTGGTATGGCTTCCTTTTTCTCCTTTT 780

GTTGCTCAAAGCATACATTTGTTTGGGTGGAAAAGTTAATATTAGATGTTCCAAATTTCT 840

TTGCACCACACTTCTTCGTTCTTTAAATTTTTGCTTCAGTTTTTTTACTTCATCAATGTC 900

TCCAAGAGCATATTGTAAATACAATTTTCTTATCTTTTCTTTAGAGCACACTGGCGGCCG 960
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FIG. 2. **Gene and protein sequences for *S. pombe* IPP isomerase.** DNA from the host vector pDB20 is in *bold*. Start and stop codons are in *italics*.

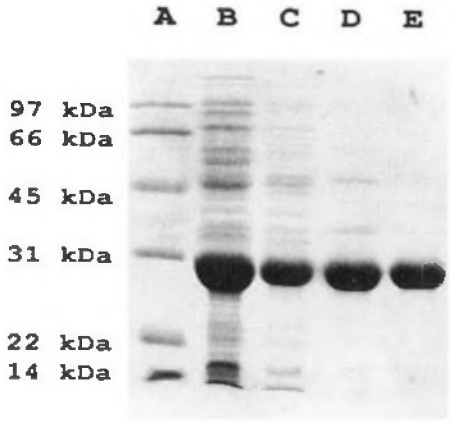


FIG. 3. **SDS-polyacrylamide gel of steps in the purification of recombinant *S. pombe* IPP isomerase.** *Lane A*, molecular mass standards; *lane B*, cell-free extract; *lane C*, after chromatography on DE52; *lane D*, combined "early" fractions after chromatography on phenyl-Sepharose; *lane E*, combined "late" fractions after chromatography on phenyl-Sepharose.

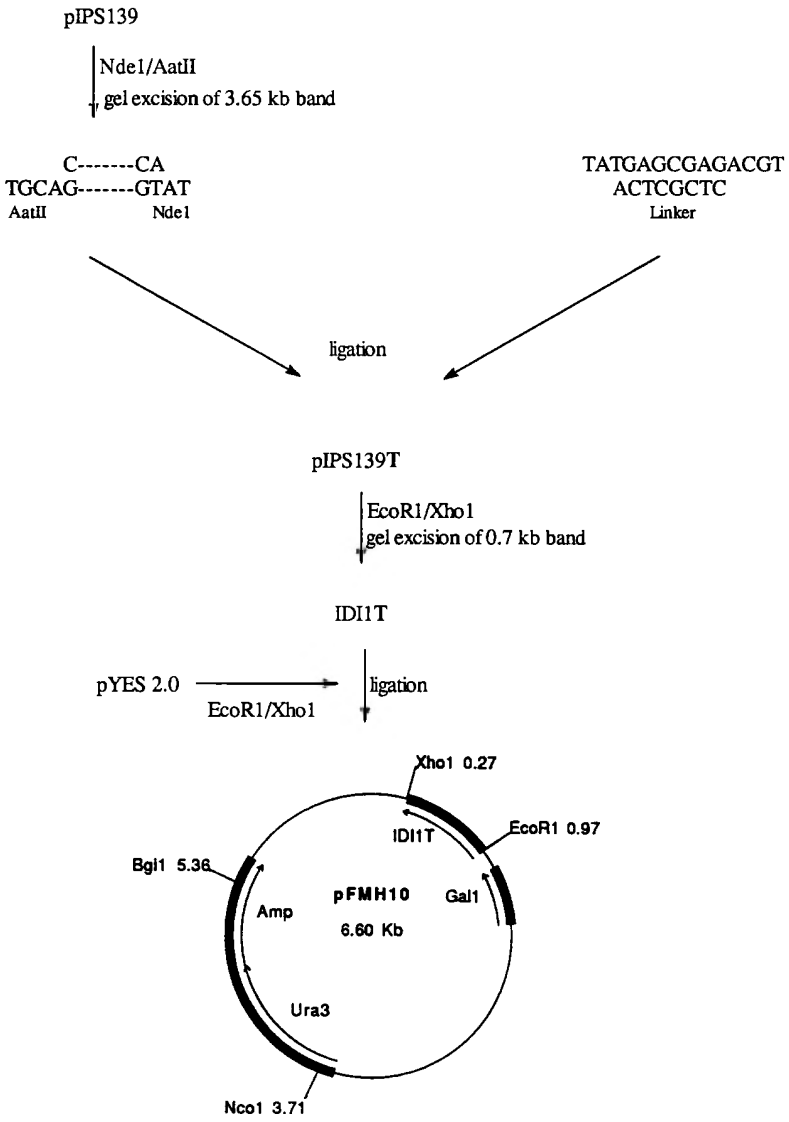
bined. $(NH_4)_2SO_4$ (1.5 M), 100 mM potassium phosphate, 10 mM BME, pH 7.0, was added to the combined fractions, while stirring on ice, until a final concentration of 1.2 M $(NH_4)_2SO_4$ was reached. The solution was then loaded at 4 °C onto a 1.5 × 30-cm phenyl-Sepharose column preequilibrated with 1.2 M $(NH_4)_2SO_4$, 100 mM potassium phosphate, 10 mM BME, pH 7.0 (buffer C). Purification was achieved by running a 100-ml linear gradient to 100% buffer D containing 100 mM potassium phosphate, 10 mM BME, pH 7.0.

Truncation of S. cerevisiae IPP Isomerase—S. cerevisiae IDI1 was isolated from a *NdeI/AatII* double digestion of pIPS139 (18) by electrophoresis on a 0.8% agarose gel. The 3.65-kb DNA fragment was excised, purified by GeneClean, and recircularized with a synthetic double-stranded oligonucleotide, containing the necessary complementary ends to give pIPS139T. Digestion of the plasmid with *Eco*RI and *Xho*I gave a DNA fragment containing yeast *IDI1* lacking the 5′ sequence encoding 45 amino-terminal amino acids. The 0.7-kb DNA fragment was subcloned into the *Eco*RI and *Xho*I sites of the yeast expression vector pYES2.0 to give pFMH10.

RESULTS

α-AA Selection/Counterselection of S. cerevisiae FH2-5b Transformants—The *S. pombe* library used in these experiments was constructed by cloning poly(dT)-primed cDNA into the unique *Bst*XI site of pDB20, a yeast-*E. coli* shuttle vector with a *URA3* selectable marker and a yeast *ADH1* promoter (20). More than 40,000 colonies were obtained upon transformation of yeast strain FH2-5b and selection for uracil, leucine, and lysine prototrophy. The transformants were subjected to selection/counterselection as described by Sikorski and Boeke (24). Haploid strain FH2-5b had a disrupted chromosomal copy of *IDI1* which was complemented by an episomal copy of the wild-type gene on pFMH1 (15). pFMH1 also contained *LYS2* and the *LYS2* gene product produced a toxic semialdehyde when FH2-5b was grown on medium containing α-AA (34). These conditions selected against cells that did not lose pFMH1. Thus, double transformants of FH2-5b containing pFMH1 and a plasmid from the cDNA library lost pFMH1 upon treatment with α-AA (24). However, since *IDI1* is an essential single-copy gene in yeast, the loss of pFMH1 was lethal unless the *idi1* mutation was complemented by a functional isomerase gene in the cDNA library. Of the >40,000 colonies transformed with *S. pombe* cDNA, only 23 survived counterselection with α-AA (24).

Plasmid DNA from a random selection of 11 of the 23 survivors was used to transform *E. coli* strain DH5α. Restriction analysis of *Bgl*I fragments from *E. coli* plasmid DNA transformants gave the patterns shown in Fig. 1. Host vector pDB20



SCHEME 2. **Construction of yeast expression vector pFMH10 containing the truncated *S. cerevisiae* IPP isomerase gene (*IDI*1T).**

gave bands at 1.5, 2.0, and 3.7 kb. In contrast, DNA from the library gave distinct bands at 1.5 and 3.7 kb, but the band at 2.0 kb was replaced by a smear between 2.0 and 3.5 kb. Four of the 11 survivors selected for further study contained plasmids that gave identical *Bgl*I restriction patterns with bands at 1.5, 3.0, and 3.7 kb. Plasmids from four of the survivors that did not give bands at 1.5 and 3.7 kb were subsequently treated with *Sal*I/*Nco*I and found to give a band at 0.5 kb corresponding to *Nco*I and *Sal*I restriction sites in wild-type *IDI*1.

*Complementation of Disrupted IDI*1—The diploid yeast strain FH1 (15), heterozygous for the *idi*1::*leu*2 disruption, was transformed with each of the four positive plasmids identified by restriction analysis, each corresponding to a different survivor of α-AA counterselection. Sporulation and dissection of the four transformants gave viable spores that were ura$^+$ and leu$^+$, indicating that the *URA*3 marker of the pDB20-derived plasmid had segregated with the *LEU*2 marker of the gene disruption. Mating type analysis showed that the uracil and leucine prototrophs were haploid. Thus, all four plasmids contained DNA that was able to complement the disrupted IPP isomerase gene.

Characterization of S. pombe IDI1 cDNA—S. pombe cDNA was removed from the four positive clones by restriction with *Hin*dIII and ligated into pBluescript II SK(+). Partial DNA sequencing experiments indicated that all four inserts were identical. A single plasmid was selected and sequenced. An orf beginning with a methionine codon was found 8 base pairs downstream from the *Bst*XI cDNA insertion site. The nucleotide sequence of the *S. pombe* IPP isomerase orf and flanking DNA is shown in Fig. 2. The orf encodes a protein of 227 amino acids with a predicted mass of 26,864 daltons. Although the *S. pombe* protein has a high degree of similarity to *S. cerevisiae* IPP isomerase, the smaller *S. pombe* enzyme lacks 52 amino acids found at the amino terminus of *S. cerevisiae* IPP isomerase.

Expression of S. pombe IDI1 and Purification of IPP Isomerase—Kunkel mutagenesis was used to introduce a *Nde*I site at the start codon and a *Sal*I site just downstream from the stop codon of the *S. pombe* orf. The 0.7-kb *Nde*I/*Sal*I fragment was cloned into pARC306N as described for the *S. cerevisiae* gene (18). The resulting plasmid, pFMH6, was used to transform *E. coli* strain JM101. Cultures of JM101/pFMH6 in M9/CAGM medium were grown at 37 °C, and plasmid-directed protein synthesis was induced by the addition of nalidixic acid. *S. pombe* IPP isomerase was purified from cell-free crude extracts in two steps by ion exchange chromatography on DE52-cellulose and by hydrophobic interaction chromatography on phenyl-Sepharose as described for yeast protein (18). This procedure gave enzyme that was greater than 95% pure as judged by

SDS-polyacrylamide gel electrophoresis (see Fig. 3). The specific activity of the pure *S. pombe* IPP isomerase (20 units/mg) is similar to that of the *S. cerevisiae* enzyme (18).

Truncation of S. cerevisiae IPP Isomerase and Functional Complementation of Disrupted IDI1—Deletion of the 45 amino-terminal amino acids in *S. cerevisiae* IPP isomerase was accomplished by removal of the 143-base pair fragment between the *Nde*I site in the start codon and the downstream *Aat*II site to give a protein of similar size to the *S. pombe* IPP isomerase and the putative human enzyme. As outlined in Scheme 2, pIPS139 (18) was restricted with *Aat*II and *Nde*I. The 3.65-kb band was purified by agarose gel electrophoresis and ligated with a synthetic linker to close the incompatible ends and maintain the proper reading frame. The region corresponding to the orf for IPP isomerase in the truncated plasmid pIPS139T was sequenced.

The truncated *S. cerevisiae IDI*1 gene was expressed in *E. coli*. The 0.8-kb *Nde*I/*Sal*I fragment from pIPS139T was ligated into pARC306N to create pFMH5. *E. coli* JM101/pFMH5 transformants were used to inoculate M9/CAGM cultures grown at 37 °C. Cell-free crude extracts from JM101/pFMH5 had 5-fold increased levels of isomerase activity above those from JM101 controls containing pARC306N without the insert (28). Analysis of low speed supernatants and pellets of cell-free extracts from *E. coli* JM101/pFMH5 by SDS-polyacrylamide gel electrophoresis indicated that a substantial portion of the truncated protein segregated with the pellet.

Rather than optimizing expression in *E. coli*, we decided to determine if the truncated yeast gene would complement disrupted *IDI*1 to give a viable *S. cerevisiae* strain. The truncated orf for yeast IPP isomerase was removed from pIPS139T as a 0.7-kb fragment by digestion with *Eco*RI/*Xho*I and cloned into the yeast expression vector pYES2.0 to produce pFMH10. Diploid *S. cerevisiae* strain FH1, an *idi*1::*leu*2 heterozygote, was transformed to uracil prototrophy. Sporulation medium (23, 35) was inoculated with isolated transformants and incubated at 30 °C for 5 days. The resulting asci were dissected on a YEPD plate and incubated at 30 °C. Haploid spores able to grow without uracil and leucine were identified by replica plating as shown in Fig. 4. Thus, the truncated form of *S. cerevisiae* IPP isomerase, although unstable, had sufficient activity to complement disrupted *IDI*1.

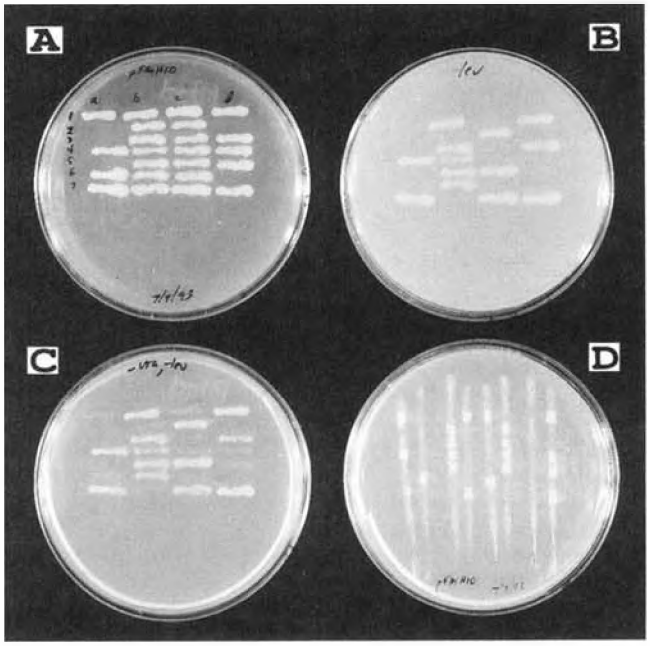


FIG. 4. **Plates from test for complementation of disrupted *IDI*1 by the truncated *S. cerevisiae* IPP isomerase gene contained in pFMH10.** *Plate A*, YEPD master plate with restreaks of dissected asci. *Plate B*, master plate replica plated to leu$^-$ medium. *Plate C*, master plate replica plated to ura$^-$, leu$^-$ medium. *Plate D*, mating type analysis showing spores able to mate by a patch of growth at cross-streaked intersections and thus likely to be haploid.

DISCUSSION

The conversion of IPP to DMAPP catalyzed by IPP isomerase is an essential step in the biosynthesis of all isoprenoids. Although impure preparations of the enzyme have been studied from a wide variety of organisms, the only proteins purified to homogeneity are those from *S. cerevisiae* (13, 14) and *Claviceps purpurea* (11). The gene for the *S. cerevisiae* enzyme, *IDI*1, was isolated from a genomic library by colony hybridization using oligonucleotide probes designed from the amino-terminal sequence of the protein. In yeast, *IDI*1 is an essential single-copy gene (15).

Because of the unusual mechanism of action of IPP isomerase and its widespread occurrence in nature, we wanted to isolate genes for the enzyme from a variety of organisms for comparative studies. Although the approach we used to isolate the yeast gene could be applied to other organisms, in some cases isomerase activity is very low and the protein difficult to purify (7, 14). An approach based on complementation of an IPP isomerase deficiency appeared to be more practical. Prior to our disruption of *IDI*1 in *S. cerevisiae* (15), there were no reports of an IPP isomerase mutant suitable for complementation experiments. We decided to use the disrupted yeast strain FH1, a heterozygote for the *idi*1::*Leu*2 disruption, for complementation experiments using the plasmid shuffle technique to isolate conditional lethal mutations (24).

Haploid *S. cerevisiae* strain FH2-5b (*MATα idi*1::*LEU*2 pRS317:*IDI*1) was constructed by transformation of diploid strain FH1 followed by sporulation and isolation of the appropriate segregant. Transformation of FH2-5b with the *S. pombe* cDNA library followed by counterselection with α-AA gave 23 survivors from a total of 40,000 cotransformants. Four of the 23 were cDNA clones for *S. pombe IDI*1. The remaining survivors were not fully characterized but could have arisen from mutations resulting in inactivation of *LYS*2 or recombination events that separated *IDI*1 from *LYS*2 in pFMH1. In any event, the frequency of false positives was only slightly greater than that for complementation by the cDNA for *S. pombe* IPP isomerase. This technique should be applicable for any heterologous library that can direct synthesis of an active IPP isomerase in yeast.

The orf for *S. pombe* IPP isomerase encoded a protein that lacked approximately 52 amino acids at the amino terminus of the *S. cerevisiae* enzyme. Recombinant *S. pombe* isomerase produced in *E. coli* was a stable protein whose specific activity was similar to that of the longer yeast enzyme. Although a truncated mutant of yeast isomerase lacking 45 amino acids at its amino terminus was less stable than wild-type enzyme, cell-free homogenates of *E. coli* clones had high levels of isomerase activity, and FH1 transformants containing the gene for the truncated enzyme allowed complementation of disrupted *IDI*1 following sporulation and dissection.

As shown in Fig. 5, alignment of the amino acid sequences for the two fungal isomerases reveals several regions of high similarity. In particular, Cys^{139} in the *S. cerevisiae* enzyme and the corresponding residue, Cys^{87}, in *S. pombe* isomerase are located in identical sequences of 11 amino acids. Likewise, Glu^{207} and Glu^{152} in the respective enzymes are found in regions containing 11 conserved amino acids. These cysteine and glutamate residues are important components of the catalytic site of *S. cerevisiae* IPP isomerase (16–18) and presumably fulfill

```
ISOMPOMBE   MSSQQEKK-------------------------------------------      8
ISOMSCEREV  MTADNNSMPHGAVSSYAKLVQNQTPEDILEEFPEIIPLQQRPNTRSSETS     50
ISOMHUMAN   MMPEINT--------------------------------------------      7
            * .. ..

ISOMPOMBE   ----------DYDEEQLRLMEEVCIVVDENDVPLRYGTKKECHLMENINK     48
ISOMSCEREV  NDESGETCFSGHDEEQIKLMNENCIVLDWDDNAIGAGTKKVCHLMENIEK    100
ISOMHUMAN   ---------NHLDKQQVQLLAEMCILIDENDNKIGAETKKNCHLNENIEK     48
                      *..*..*..* **..* .*   .  .*** *** ***.*

ISOMPOMBE   GLLHRAFSMFIFDEQNRLLLQQRAEEKITFPSLWTNTCCSHPLDVAGERG     98
ISOMSCEREV  GLLHRAFSVFIFNEQGELLLQQRATEKITFPDLWTNTCCSHPLCIDDELG    150
ISOMHUMAN   GLLHRAFSVFLFNTENKLLLQQRSDAKITFPGCFTNTCCSHPLSNPAELE     98
            ********.*.*.... ******...*****. .*********   .* .

ISOMPOMBE   --NTLPEAVEGVKNAAQRKLFHELGI-QAKYIPKDKFQFLTRIHYLAPST    145
ISOMSCEREV  LKGKLDDKIKGAITAAVRKLDHELGIPEDETKTRGKFHFLNRIHYMAPSN    200
ISOMHUMAN   ESDAL-----GVRRAAQRRLKAELGIPLEE-VPPEEINYLTRIHYKAQSD    142
              . *     *.  ** *.*  ****  ..  . .....*.**** *.*.

ISOMPOMBE   GAWGEHEIDYILFFK--GKVELDINPN--EVQAYKYVTMEELKEMFSDPQ    191
ISOMSCEREV  EPWGEHEIDYILFYKINAKENLTVNPNVNEVRDFKWVSPNDLKTMFADPS    250
ISOMHUMAN   GIWGEHEIDYILL----VRKNVTLNPDPNEIKSYCYVSKEELKELLKKAA    188
            . **********.      . ....**.  *.... .*. ..**... ..

ISOMPOMBE   ---YGFTPWFKLICEHFMFKWWQDVDHASKFQ-DTLIHRC-    227
ISOMSCEREV  ---YKFTPWFKIICENYLFNWWEQLDDLSEVENDRQIHRML    288
ISOMHUMAN   SGEIKITPWFKIIAATFLFKWWDNLNHLNQFVDHEKIYR-M    228
                .*****.* . ..*.**..... ..   .  *.*

Identity: 94 ( 32.3%)  Similarity: 79 ( 27.1%)
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FIG. 5. **Sequence alignment of the three eukaryotic IPP isomerases: *S. pombe* (ISOMPOMBE), *S. cerevisiae* (ISOMSCEREV), and human (ISOMHUMAN).** Identity is indicated by *, and similarity is indicated by . . The active site residues identified in *S. cerevisiae* IPP isomerase, Cys^{139} and Glu^{207} (see Ref. 16), are conserved in all three isomerases and are shown in boldface italic.

identical roles.

Recently Xuan *et al.* (19) isolated a human cDNA clone by differential hybridization of a human promyelocytic leukemia cell line with and without treatment with phorbol esters. Although the encoded protein was not expressed and characterized biochemically, its amino acid sequence showed a high degree of similarity to *S. cerevisiae* IPP isomerase. In addition, the human protein also lacked the same amino-terminal region missing in the *S. pombe* enzyme. As shown in Fig. 5, the human and *S. pombe* proteins are of almost identical length and share the same highly conserved regions with the *S. cerevisiae* enzyme, including the identical stretches surrounding the active site cysteine and glutamate.

The *S. cerevisiae* strain FH2-5b was constructed to allow screening of the heterologous libraries for DNA encoding IPP isomerase by complementation through a selection/counterselection protocol. We used the approach to screen a *S. pombe* cDNA library in the presence of α-AA and isolated a clone for IPP isomerase. We anticipate that selection/counterselection with FH2-5b should be generally useful for isolating plasmids that can direct synthesis of functional IPP isomerase in genomic and cDNA libraries.

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